

A New Type of Lesion Associated with Severe Fur Damage in Canadian Ranch Foxes and an Investigation of Possible Causes

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ABSTRACT

In the silver fox, as in its wild ancestor, the red fox (*Vulpes vulpes* L.), the annual growing phase (anagen) of guard hair follicles occupies at least four months. Severe damage to the hair coat near the end of this growing period was reported in 1985 on many ranches in New Brunswick and Nova Scotia. A histological analysis of serial sections of skin biopsies showed a marked increase in nuclear aberrations in the hair matrix of anagen guard hair follicles. These nuclear aberrations indicated that cells were undergoing apoptosis, a controlled form of cell death. Tissues from affected and unaffected foxes for histological and toxicological analysis, as well as other data, were obtained during visits to 26 ranches in 1986 and 34 ranches in 1987. Histological sections of the 1987 skin samples showed the mean percentage of nuclear aberrations in 43 unaffected foxes to be 0.08 ± 0.01 (SEM), while that for 49 affected foxes was 0.51 ± 0.23 . The four foxes with the most severe coat damage also had the highest incidences of guard hair matrix cells with nuclear aberrations, ranging from 20 to 100 times greater than the mean for unaffected foxes. The mitotic index of the hair matrix, which normally remains fairly constant during the hair growth phase, was similar for unaffected and affected foxes (1.83 ± 0.06 and 1.97 ± 0.07 respectively). Although our analyses of field data have not established a specific environmental factor associ-

ated with increased nuclear aberrations, the possible involvement of toxic agents in follicle damage may warrant further investigation.

RÉSUMÉ

Chez le renard argenté, ainsi que chez son ancêtre sauvage le renard roux (*Vulpes vulpes* L.) la phase de croissance annuelle (anagène) des follicules des poils de jarre dure au moins quatre mois. Une détérioration sévère du pelage vers la fin de cette phase de croissance a été rapportée en 1985 dans plusieurs élevages au Nouveau-Brunswick et en Nouvelle-Écosse. Une étude histologique de sections sériées des biopsies cutanées démontrait une augmentation marquée des aberrations nucléaires dans la matrice des poils de jarre en phase anagène. Les aberrations nucléaires indiquaient la présence d'apoptose au niveau des cellules, une forme contrôlée de mort cellulaire. Des tissus prélevés à partir de renards affectés et non-affectés ont été obtenus lors de la visite de 26 élevages en 1986 et 34 élevages en 1987 pour fin d'analyses histologiques et toxicologiques.

Les coupes histologiques des échantillons cutanés prélevés en 1987 ont démontré un pourcentage d'aberrations nucléaires de $0,08 \pm 0,01$ (SEM) chez 43 renards non-affectés tandis qu'il était de $0,51 \pm 0,23$ chez 49 renards affectés. Les quatre renards dont le pelage était le plus endommagé avaient aussi la plus haute incidence d'aberrations nucléaires de cellules de

la matrice des poils de jarre, variant de 20 à 100 fois plus que la moyenne chez les renards non-affectés. L'index mitotique de la matrice du poil, qui demeure normalement passablement constant durant la phase anagène, était semblable pour les renards non-affectés et affectés ($1,83 \pm 0,06$ et $1,97 \pm 0,07$, respectivement).

Quoique les analyses des données recueillies sur le champ n'aient pas établi un facteur environnemental associé à une augmentation des aberrations nucléaires, l'implication possible d'agents toxiques dans le dommage des follicules pileux devrait justifier d'autres investigations. (Traduit par Dr Manon Paradis)

INTRODUCTION

A sudden onset of severe coat damage and hair loss in silver foxes on many ranches in the maritime provinces of New Brunswick and Nova Scotia, shortly before pelting time towards the end of 1985, prompted the investigation reported in this paper. We were asked by a veterinarian with the Ontario Ministry of Agriculture and Food to examine fur samples and histological sections of skin from three silver foxes with damaged coats. In our preliminary investigations, microscopic spaces were found within the fragile guard hairs taken from these foxes. In the skin sections, the guard hair follicles which were in the anagen or growing phase showed changes in the "hair matrix" cells which give rise to the hair. A large proportion of these matrix cells

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showed nuclear aberrations, indicating various stages in apoptosis, a process by which individual cells within a healthy population can undergo programmed cell death (1). Apoptosis can be distinguished histologically from necrosis, where an entire group of adjacent cells undergoes functional breakdown in response to an unfavourable environment (2).

At the time when fur damage was noted, the foxes were near the end of the four to five month annual growth period for guard hair follicles, and approaching the end of the seasonal growth of all follicles (3). Normally, in both the silver fox and its wild ancestor, the red fox (*Vulpes vulpes* L.), all these follicles would proceed to undergo some remodelling (catagen phase), then pass quickly into a resting (telogen) phase, during which the fibers are retained in the follicle until the spring molt (3,4). During catagen and telogen, no damage occurs in the cells of the hair matrix in other species that have been studied extensively; they simply cease to proliferate (5-7). Although the timing of growth cycles in both types of follicles can be altered in foxes and other fur-bearing animals by varying the hours of exposure to daylight (8) and also by altering the thyroid or other hormone levels (9), this is accomplished without damage to hair matrix cells (6). However, both ionizing radiation and certain chemicals in the environment have been shown to be genotoxic, causing apoptosis and/or chromosome breakage in the proliferating hair matrix cells of anagen follicles of mice and humans (10-12), as well as similar effects in other tissues such as colonic epithelium (13) and mammary epithelium (14). Apoptosis occurring in the hair matrix cells results in either narrowing of the hair shaft and brittle hairs, or total cessation of hair growth and sudden loss of hairs (10). We therefore hypothesized that an environmental genotoxic agent might have induced the hair damage observed in the Maritime foxes. To explore this possibility, we collected material for laboratory study from selected ranches in the Maritime provinces and in southern Ontario in the fall of 1986 and 1987, including ranches where the

hair damage problem was reported and those where no problem was perceived.

MATERIALS AND METHODS

Visits were made to 20 ranches in New Brunswick, Nova Scotia and Prince Edward Island in the period November 13-26, 1986. One or two skin samples were obtained with a 6 mm biopsy punch from the hip region of two to four foxes on each ranch and were immediately placed in neutral buffered formalin. Where possible, the collection from each ranch included at least one skin biopsy from an animal considered by the rancher to have a hair growth problem ("affected") and one not showing a hair problem ("unaffected"). Fur samples were also collected from the 46 animals biopsied. Between December 3 and December 5, 1986, similar visits were made to six ranches in southern Ontario, where 13 foxes were sampled.

In the period October 2-20, 1987, visits were made to 31 ranches in the Maritime provinces. Eighteen of these ranches were among those sampled in 1986. The procedure was the same as before, but there was an increase in the average number of foxes sampled per ranch, with a total of 100 animals. As it was earlier in the growing season and the fur was not yet at its prime, postcards were left with ranchers to send follow-up reports of each fox's condition as priming occurred. On October 30, 1987, three southern Ontario ranches were visited, two of these being among those visited in 1986.

In both years the foxes sampled included several color mutants in addition to the standard silver foxes. A questionnaire completed by each owner in each year provided information on numbers of animals affected, fur quality, management practices, diet, drinking water, type of bedding, illness and medication, and on pesticide use on the ranch and in the locality. Information was also obtained about government-licensed pesticide spraying programs carried out in 1986 and 1987.

HISTOLOGICAL STUDIES

All skin samples were trimmed under a dissecting microscope in preparation for sagittal sectioning through the long axes of the follicles of guard hairs and fur fibers. The trimmed samples were embedded in paraffin and sectioned serially in the desired direction at a thickness of 5 or 7 μ m. Most of the sections were stained by the Feulgen method and counterstained with Fast Green, to facilitate the counting of mitoses and nuclear aberrations, but some sections from each block were stained with hematoxylin and eosin for better definition of cell boundaries and possible cytopathological changes. The stage in the growth cycle of every guard hair and some fur fibers was recorded for each of the 50-600 sections from each skin sample. Sections containing guard hair follicles in the anagen phase which were cut in the desired midsagittal or parasagittal planes and included dermal papillae, were selected for scoring. Adjacent sections were excluded to avoid the possibility of counting a cell twice. The scoring was done by a trained observer with no knowledge of any previous evaluation of the animals or sections. In the hair matrix area of each guard hair follicle, counts were made of the total number of cells, the number of mitotic cells and the number of nuclear aberrations. For the purpose of these counts, the hair matrix area was deemed to include all epithelial cells of the hair bulb, from the base of the bulb to a line two cells above the tip of the dermal papilla (Fig. 1). An isolated, condensed chromatin fragment in the hair matrix area was counted as one nuclear aberration, and a cluster of fragments of condensed chromatin which appeared to be derived from a single cell was also counted as one nuclear aberration. A minimum of 1500 cells counted per biopsy was established for the calculation of mitotic index and nuclear aberration index.

TOXICOLOGICAL ANALYSES

During the 1986 pelting, some additional tissue samples were collected from a subgroup of the biopsied foxes at necropsy. The ranches in southwestern Nova Scotia were the focus of this aspect of the study. The samples included liver, eyes and subcutaneous

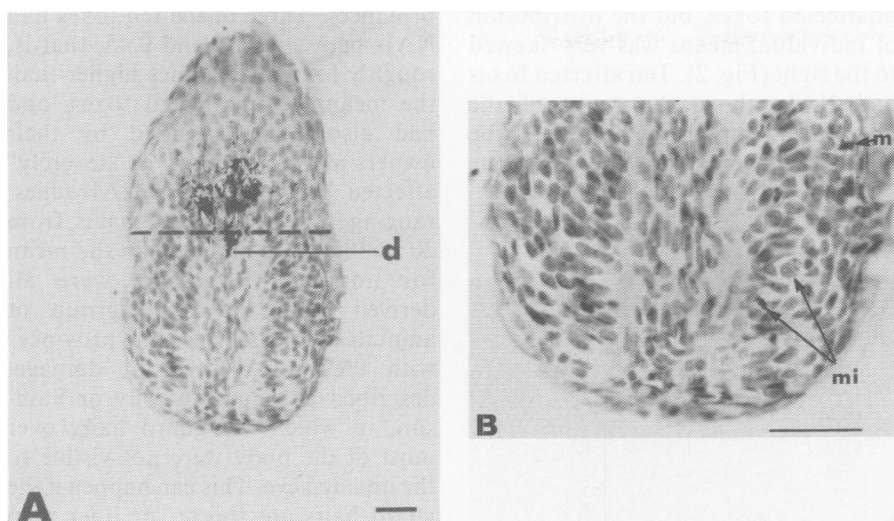


Fig. 1. A near-sagittal section through the base of a guard hair follicle in skin from a fox which was "unaffected" when sampled in 1987. A. The dashed line indicates the upper limit of cells included in the count of hair matrix cells. Immediately above the tip (d) of the paler-staining dermal papilla, several dendritic melanocytes containing black melanin granules are seen. A few melanin granules which have been transferred to the keratinocytes are also seen. B. A higher power view of the hair matrix, showing numerous mitoses of different phases (mi) and an absence of nuclear aberrations in this section. The NAI for this fox was 0.13 in 1986 and 0.04 in 1987. Feulgen, Fast Green. Scale bars indicate 100 μ m.

fat. Liver samples fixed in buffered formalin and eye samples fixed in Bouin's fluid were sent for histopathological analysis to the Pathology Department at the Ontario Veterinary College. Fat samples were frozen and sent to the Pesticide Residue Laboratory of the Ontario Ministry of Agriculture and Food, where they were assayed for commonly used herbicides and insecticides.

STATISTICAL ANALYSIS

Student's *t*-test was used to compare the mean nuclear aberration indexes of foxes considered by owners to be affected and of foxes considered to be unaffected by fur damage. To calculate a relative risk of various diets, and management and environmental factors contributing to fur damage, the odds ratio method (15) was used.

RESULTS

REPORTS BY RANCHERS ON FUR PROBLEMS DURING PRIMING IN 1986 AND 1987

In 1986 only two of the 20 ranchers visited in the Maritime provinces reported no fur problems at the time of the visit, which was towards the end of the priming period. On the remaining

18 ranches the number of foxes showing damage ranged from "several" to "100%". Damage was variously described in the following terms: cowlicks, clips, brittle guard hair with broken tips, abnormal patches, rough coat, thin tail fur, etc. In our selected sample of 46 foxes from the 20 ranches, 41 were considered to be affected to some degree.

Of 12 ranchers consulted in Ontario in 1986, only two reported a problem. One of these two had some "scruffy-looking" animals, and the other reported foxes in which the guard hairs were not visible to the unaided eye. Thirteen foxes, including three of those said to be affected, were sampled from six ranches in Ontario.

Most of the 31 Maritime ranchers visited in 1987 considered that 1987 was a better year than 1986, with less fur damage, although some problems still existed. Two ranchers thought they had more damage than in 1986. Of 100 foxes in the selected sample, 53 were said to be affected. The foxes were sampled about one month earlier in the season than in 1986, and therefore the owners regarded their assessment of individual animals as more tentative. However, their follow-up reports showed that 41 out of 46 reassessments made at pelting time were unchanged, and five were changed from "affected" to "unaffected". Descriptions of the fur problems were similar to those given in 1986, but also included terms such as "spiky", "wavy" and "singd", the last referring to the most severe damage, hair that is short and frizzy (or wiry, or curly) over the entire body. "Woolly" and "Samson" fur were alternative descriptions of the most severely affected foxes. Broken tips and narrowing shafts were found in the guard hairs from these fur samples.

Only three ranches were visited in Ontario in 1987, and three of the eight foxes sampled were said to be affected. They were described respectively as "weak hair", "woolly" and "did not fur properly".

SKIN SAMPLING AND HISTOLOGICAL EXAMINATION

In 1986, November was chosen as a suitable month for sampling skin from the hip to obtain guard hair follicles in late anagen [on the basis of the year-round observations of silver fox pelage in New York State by Bassett and Llewellyn (3)], but this proved to be a little too late for two thirds of the Canadian foxes sampled. As a result

TABLE I. Mean values (\pm SEM) for mitotic index and nuclear aberration index in "unaffected" and "affected" foxes

Year	Group	No. of foxes	Mitotic index	Nuclear aberration index
1986	Unaffected	2	1.29 \pm 0.26	0.05 \pm 0.01
	Affected	7	1.45 \pm 0.14	0.15 \pm 0.04
1987	Unaffected	43	1.83 \pm 0.06	0.08 \pm 0.01
	Affected	49	1.97 \pm 0.07	0.51 \pm 0.23
	Affected (adjusted) ^a	45	2.02 \pm 0.07	0.10 \pm 0.01

^aFour foxes with a "singd" or "Samson" appearance and unusually high mean nuclear aberration indexes (>1.00) were excluded

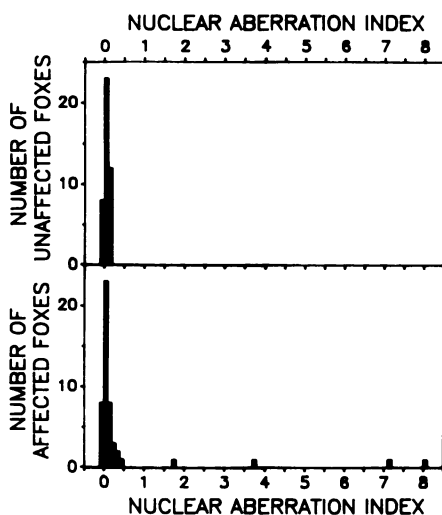


Fig. 2. Histograms showing the distribution of nuclear aberration indexes (NAI) for "unaffected" and "affected" foxes which were sampled in 1987 and successfully scored. Ten foxes in the affected group had higher NAIs.

of this and technical difficulties, only nine of the 59 foxes had sufficient anagen follicles to yield the 1500 hair matrix cells deemed necessary for statistical analysis of the mitotic index (MI: percentage of cells in a population which are in mitosis) and nuclear aberration index (NAI: percentage of cells in a population showing nuclear aberrations). In 1987, the sampling in October resulted in successful analysis from 92 of 108 foxes. Table I summarizes the results obtained for 1986 and 1987 from animals judged to be "unaffected" and those considered to be "affected" to any degree.

In sections from the affected and unaffected foxes sampled in 1986, the general histological appearance of skin and hair follicles was similar. The mean MI for hair matrix of guard hair follicles in anagen was also similar in the two groups, but the mean NAI was three times higher in the affected group. However, the numbers of animals were too low for testing of statistical significance.

In the 1987 samples, there was no significant difference between the mean MI for 43 unaffected foxes, and that for 49 foxes said to be affected to some degree. The mean values for both groups were slightly higher than those for the 1986 groups (Table I). The mean NAI of affected foxes was more than six times higher than that of

unaffected foxes, but the distribution of individual means was very skewed to the right (Fig. 2). Ten affected foxes had NAIs above the range of the unaffected animals (0.00-0.19). Nine of the ten were from the Maritime

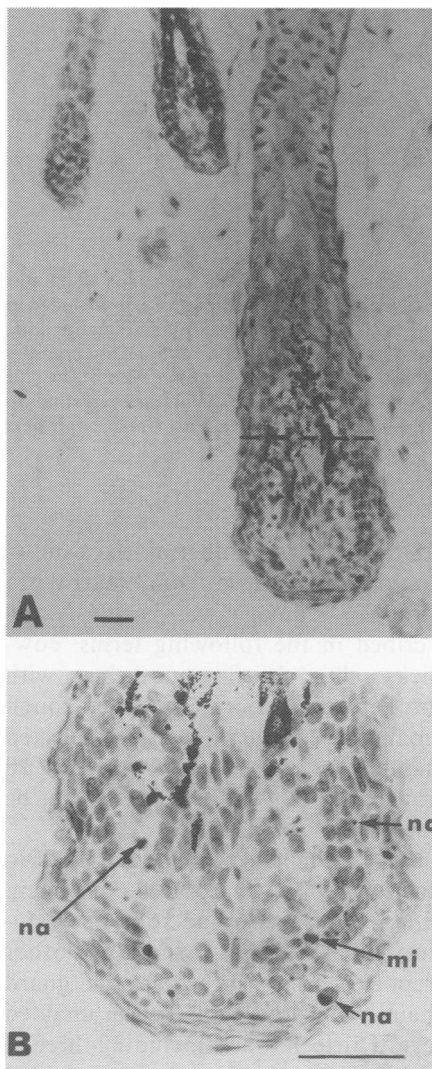


Fig. 3. A sagittal section through hair follicles in skin from a fox showing severe coat damage ("Samson" appearance) when sampled in 1987. A. A guard hair follicle and a fur follicle in anagen, the former showing nuclear aberrations. The dashed line indicates the upper limit of cells included in the count. B. A higher power view of the guard hair matrix, showing only one mitotic figure (mi), and numerous nuclear aberrations (na) as irregular bodies showing varying intensities of Feulgen staining. The NAI for this fox was 7.2. In the stained sections the contrasting colors of condensed chromatin (deep red) interphase nuclei (purple-blue), cytoplasm (green) and melanin (black or brown) make these structures very easy to recognize. Feulgen, Fast Green. Scale bars indicate 100 μ m.

provinces. Three of the ten foxes had NAIs between 0.35 and 0.45, that is, roughly four to six times higher than the mean for unaffected foxes, and had also been classified by their owners as "moderately" or "severely" affected. The four highest NAI values, ranging from 1.65 to 8.03, that is, from 20 to 100 times higher than the mean for unaffected animals, were all derived from the small group of animals from the Maritime provinces with the most severe fur damage, described as singed, woolly or Samson, in which the guard hairs over most of the body were not visible to the unaided eye. This can happen if the guard hairs are shorter or finer than usual, or if their tips are broken off. When the four highest NAI values were omitted from the analysis, the adjusted mean NAI for affected animals was 0.10 ± 0.01 , compared with 0.08 ± 0.01 for the unaffected foxes, and the difference between these two means was not significant by Student's *t*-test. Figure 3, showing a typical guard hair follicle base from one member of the most severely damaged group of foxes, may be compared with Fig. 1.

TOXICOLOGICAL ANALYSIS OF OTHER TISSUES

Liver samples collected in 1986 showed no histopathological changes. The eyes from 13 foxes, including retinal and optic nerve areas, showed normal histology. Subcutaneous fat was analyzed for pesticide residues using gas chromatography. Pesticides scanned included alachlor, metolachlor, atrazine, D-atrazine, dicamba, (2,4-dichlorophenoxy) acetic acid, other phenoxyherbicides, organochlorine insecticides and organophosphate insecticides. No pesticide residues were detected in the fat samples.

ANALYSIS OF ADDITIONAL DATA COLLECTED

The relationship of various commercial diets, water supplies, medications and potential pesticide exposures to the description of foxes as "affected" or "unaffected" has been examined for the 108 foxes sampled in 1987. No particular condition has been confirmed as having a significant odds ratio for affecting the fur. The age, sex and presence of color

mutations were also without a statistically significant effect on the fur condition in this group of animals.

DISCUSSION

This study has provided, for the first time, some data on the background level of nuclear aberrations in the hair matrix of anagen guard hair follicles in normal, healthy foxes. The mean value for NAI in unaffected foxes in 1987, 0.08 ± 0.01 SEM, was comparable with previously reported mean background levels of 0.11 ± 0.05 for the hair follicles of untreated laboratory mice (calculated from the data of Potten; 10), 0.20 ± 0.04 for laboratory mice studied by Goldberg *et al* (12) and 0.06 ± 0.03 for plucked human scalp hairs (11).

The mean mitotic indexes in the hair matrix for the unaffected and the affected groups of foxes in 1987, 1.83 and 1.97 respectively, were very similar, and the ranges were also very similar. Thus there was no evidence of interference with the maintenance of a normal cell proliferation rate in the case of the affected foxes. Other reported values of mean mitotic index are 1.36 for mouse guard hairs ("large follicles"; 10) and 0.92 for human scalp hair (11).

All the ten samples from affected foxes which showed NAIs above the range for unaffected foxes came from animals whose fur was described as moderately or severely damaged. Thus the reported fur damage in this subgroup correlates with, and may be attributed to, increased nuclear aberrations in guard hair follicles at the end of their long anagen phase. Some of these animals were reported to have had normal prime coats for one or two years before the year of sampling, and this tends to support the idea of an environmental cause of damage in the year that biopsies were taken. The four highest NAI values were as high as those reported in laboratory mice showing severe hair damage following exposure to ionizing radiation (10,12) and in mice and

humans showing damage following exposure to genotoxic chemicals (11,12,16). Thus, although a toxic agent has not yet been identified, the hypothesis of an environmental genotoxic agent is still viable, and further investigation seems to be warranted.

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